



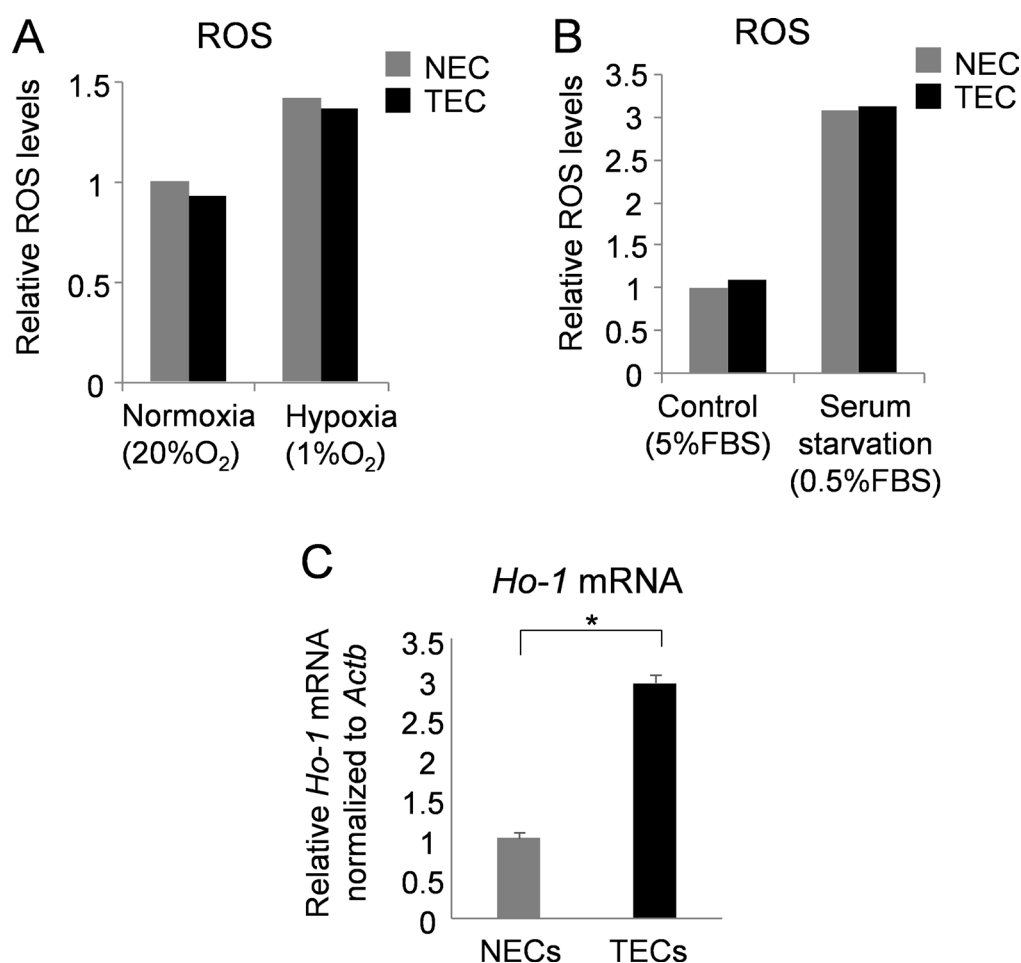
HOKKAIDO UNIVERSITY

Title	ROS enhance angiogenic properties via regulation of NRF2 in tumor endothelial cells
Author(s)	Hojo, Takayuki; Maishi, Nako; Towfik, Alam Mohammad et al.
Citation	Oncotarget, 8(28), 45484-45495 https://doi.org/10.18632/oncotarget.17567
Issue Date	2017-07-11
Doc URL	https://hdl.handle.net/2115/67123
Rights(URL)	https://creativecommons.org/licenses/by/3.0/
Type	journal article
File Information	17567-256792-1-SP.pdf, Supplementary Materials

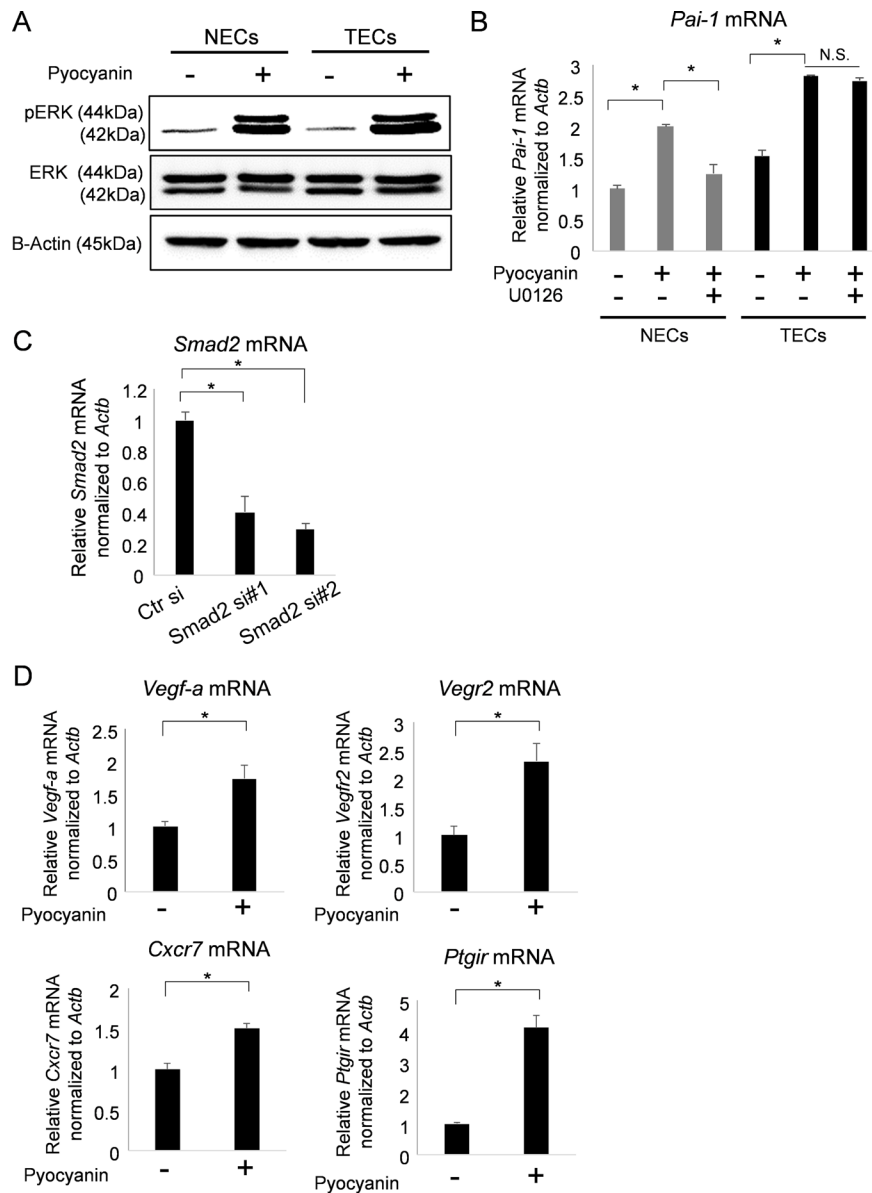


ROS enhance angiogenic properties via regulation of NRF2 in tumor endothelial cells

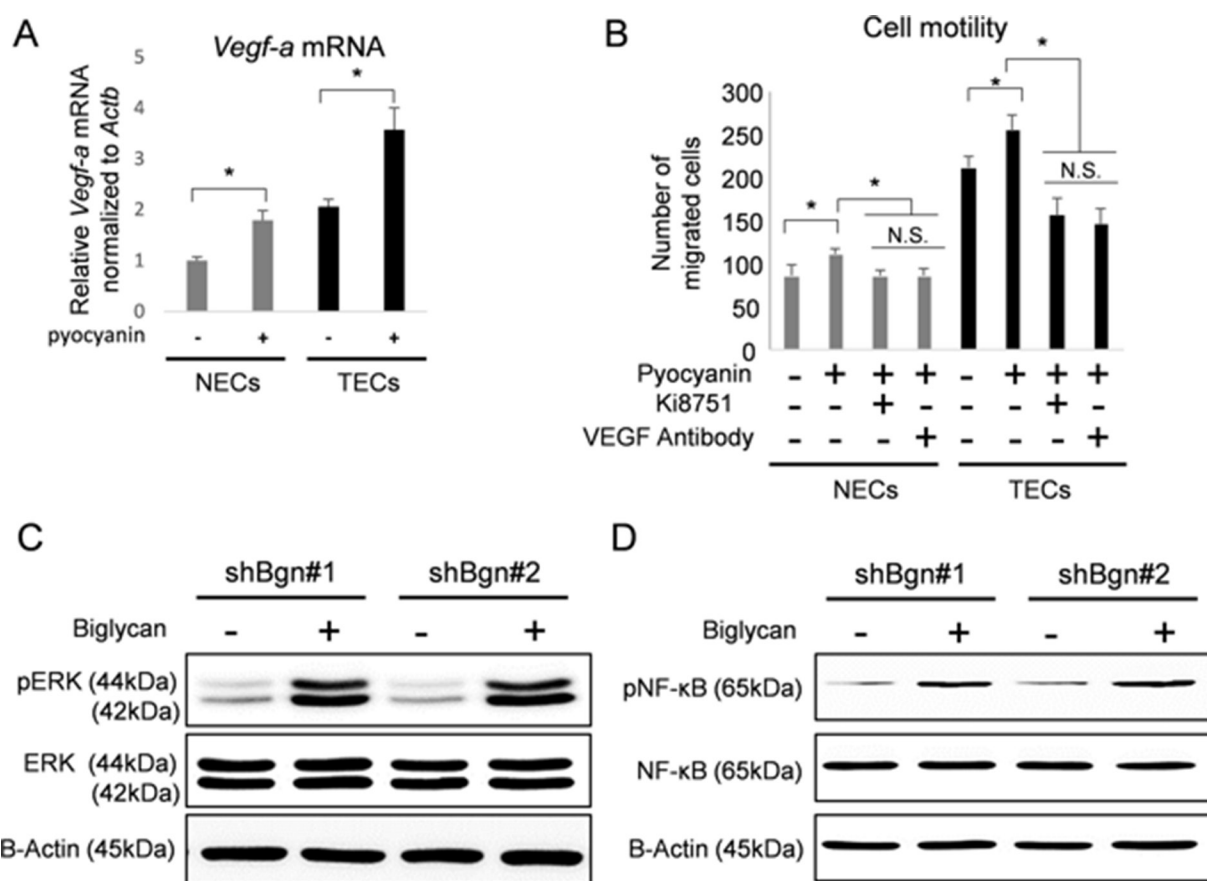
Supplementary Materials



Supplementary Figure 1: (A) Relative ROS levels in NECs (gray columns) and TECs (black columns) cultured in normoxia (20% O₂) or hypoxia (1% O₂) condition for 24 h. (B) Relative ROS levels in NECs (gray columns) and TECs (black columns) in control (medium with 5% FBS) or starved condition (medium with 0.5% FBS) for 24 h. (C) *Ho-1* mRNA in NECs and TECs were analyzed by real-time PCR. * $P < 0.01$, two-sided Student's *t*-test. Data are mean $\pm$ SD, $n = 4$ real-time RT-PCR runs.



Supplementary Figure 2: (A) Phosphorylated ERK1/2 (pERK) and total ERK1/2 (ERK) levels were analyzed in ECs stimulated with or without pyocyanin (25 μ M) by western blotting. B-Actin served as loading controls. (B) *Pai-1* mRNA in NECs and TECs with or without pyocyanin (25 μ M) or the MEK inhibitor U0126 (1 μ M) were analyzed by real-time PCR. * P < 0.01, N.S., not significant. One-way ANOVA. Data are mean $\pm$ SD, n = 4 real-time RT-PCR runs. (C) Real-time PCR confirms silenced *Smad2* mRNA levels. * P < 0.01, one-way ANOVA. Data are mean $\pm$ SD, n = 4 real-time RT-PCR runs. (D) *Vegf-a*, *Vegfr2*, *Cxcr7* and *Ptgir* mRNA expressions in TECs with or without pyocyanin treatment (25 μ M) were analyzed by real-time PCR. * P < 0.01, two-sided Student's t -test. Data are mean $\pm$ SD, n = 4 real-time RT-PCR runs.



Supplementary Figure 3: (A) *Vegf-a* mRNA in NECs and TECs with or without pyocyanin treatment (25 μ M) were analyzed by real-time PCR. $*P < 0.01$. One-way ANOVA. Data are mean $\pm$ SD, $n = 4$ real-time RT-PCR runs. (B) Cell motilities of NECs and TECs treated with pyocyanin (25 μ M) were analyzed by Boyden chamber migration assay in the presence of anti-VEGFA antibody (150ng/ml) or VEGFR2 kinase inhibitor, Ki8751 (10 nM). $*P < 0.01$, one-way ANOVA. Data are represented as mean $\pm$ SD, $n = 4$ fields. (C) Phosphorylated ERK (pERK) and total ERK (ERK) levels were analyzed in *Biglycan* knockdown TECs stimulated with BGN (1 μ g/ml) by western blotting. B-Actin served as loading controls. (D) Phosphorylated NF- κ B (pNF- κ B) and total NF- κ B (NF- κ B) in *Biglycan* knockdown TECs stimulated with BGN (1 μ g/ml) by western blotting. B-Actin served as loading controls.